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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	P-2769-US6
First Inventor or Application Identifier	STEINER, Mitchell S.
Title	METHOD FOR CHEMOPREVENTION OF PROSTATE CANCER
Express Mail Label No.	

APPLICATION ELEMENTS
See MPEP chapter 600 concerning patent application contents

ADDRESS TO: **Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231**

1. [X] * Fee Transmittal Form *(e.g., PTO/SB/17)*
(Submit an original and a duplicate for fee processing)
2. [ ] Applicant claims small entity status. See 37 CFR 1.27.
3. [X] Specification [*Total Pages* 34]
(preferred arrangement set forth below)
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to sequence listing, a table, or a computer program listing appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
4. [X] Drawing(s) *(35 U.S.C. 113)* [*Total Sheets* 6]
5. Oath or Declaration [*Total Pages* 3]
   a. [X] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 C.F.R. § 1.63(d)) *(for continuation/divisional with Box 16 completed)*
      i. [ ] DELETION OF INVENTOR(S)
      Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. [ ] Application Data Sheet. See 37 CFR 1.76
7. [ ] CD-ROM or CD-R in duplicate, large table or Computer Program *(Appendix)*
8. Nucleotide and/or Amino Acid Sequence Submission *(If applicable, all necessary)*
   a. [ ] Computer Readable Form (CRF)
   b. [ ] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] paper
   c. [ ] Statements verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

9. [ ] Assignment Papers (cover sheet & document(s))
10. [ ] 37 C.F.R. §3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
11. [ ] English Translation Document *(if applicable)*
12. [ ] Information Disclosure Statement(IDS)/PTO-1449 [ ] Copies of IDS Citations
13. [ ] Preliminary Amendment
14. [ ] Return Receipt Postcard (MPEP 5303) *(Should be specifically itemized)*
15. [ ] Certified Copy of Priority Document(s) *(If foreign priority is claimed)*
16. [X] Other: **Postcard**

17. If a **CONTINUING APPLICATION,** *check appropriate box, and supply the requisite information below and in a preliminary amendment, or in an Application Data Sheet under 37 CFR 1.76:*

[ ] Continuation [ ] Divisional [X] Continuation-in-part (CIP) of prior application No.: 09/436,208 and 09/531,472

Prior application information: *Examiner* *Group/Art Unit:*

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

18. CORRESPONDENCE ADDRESS

[ ] *Customer Number or Bar Code* *(Insert Customer No. or Attach bar code label here)* *or* [X] *Correspondence address below*

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Signature		*Date*	8 November 2000

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FEE TRANSMITTAL for FY 2001

Patent fees are subject to annual revision.

TOTAL AMOUNT OF PAYMENT ($)

Complete If Known	
Application Number	
Filing Date	
First Named Inventor	STEINER, Mitchell S.
Examiner Name	
Group / Art Unit	
Attorney Docket No.	P-2769-US6

METHOD OF PAYMENT (check one)

1. ☒ The Commissioner is hereby authorized to charge indicated fees and credit any over payments to:

Deposit Account Number: 05-0649

Deposit Account Name: Eitan, Pearl, Latzer & Cohen-Zedek

☒ Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17

☐ Applicant claims small entity status. See 37 CFR 1.27

2. ☐ Payment Enclosed:

☐ Check ☐ Credit card ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	710	201	355	Utility filing fee	710.00
106	320	206	160	Design filing fee	
107	490	207	245	Plant filing fee	
108	710	208	355	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($)710.00

2. EXTRA CLAIM FEES

			Extra Claims		Fee from Below		Fee Paid
Total Claims		-20** =		X		=	
Independent Claims		- 3** =		X		=	
Multiple Dependent				X		=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	80	202	40	Independent claims in excess of 3
104	270	204	135	Multiple dependent claim, if not paid
109	80	209	40	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet.	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	390	216	195	Extension for reply within second month	
117	890	217	445	Extension for reply within third month	
118	1,390	218	695	Extension for reply within fourth month	
128	1,890	228	945	Extension for reply within fifth month	
119	310	219	155	Notice of Appeal	
120	310	220	155	Filing a brief in support of an appeal	
121	270	221	135	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,240	241	620	Petition to revive - unintentional	
142	1,240	242	620	Utility issue fee (or reissue)	
143	440	243	220	Design issue fee	
144	600	244	300	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	710	246	355	Filing a submission after final rejection (37 CFR 1.129(a))	
149	710	249	355	For each additional invention to be examined (37 CFR 1.129(b))	
179	710	279	355	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) _____

* Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

SUBMITTED BY				Complete *(if applicable)*	
Name (Print /Type)	Mark S. Cohen	*Registration No. (Attorney/Agent)*	42,425	*Telephone*	(703) 486-0600
Signature				*Date*	November 8, 2000

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A METHOD FOR CHEMOPREVENTION OF PROSTATE CANCER

FIELD OF INVENTION

This invention relates to the chemoprevention of prostate cancer and, more particularly, to a method of suppressing or inhibiting latent prostate cancer comprising administering to a mammalian subject a chemopreventive agent and analogs and metabolites thereof. The chemopreventive agent prevents, prevents recurrence of, suppresses or inhibit prostate carcinogenesis; and treats prostate cancer.

BACKGROUND OF THE INVENTION

Prostate cancer is one of the most frequently occurring cancers among men in the United States, with hundreds of thousands of new cases diagnosed each year. Unfortunately, over sixty percent of newly diagnosed cases of prostate cancer are found to be pathologically advanced, with no cure and a dismal prognosis. One approach to this problem is to find prostate cancer earlier through screening programs and thereby reduce the number of advanced prostate cancer patients. Another strategy, however, is to develop drugs to prevent prostate cancer. One third of all men over 50 years of age have a latent form of prostate cancer that may be activated into the life-threatening clinical prostate cancer form. The frequency of latent prostatic tumors has been shown to increase substantially with each decade of life from the 50s (5.3-14%) to the 90s (40-80%). The number of people with latent prostate cancer is the same across all cultures, ethnic groups, and races, yet the frequency of clinically aggressive cancer is markedly different. This suggests that environmental factors may play a role in activating latent prostate cancer. Thus, the development of chemoprevention strategies against prostate cancer may have the greatest overall impact both medically and economically against prostate cancer.

Toremifene is an example of a triphenylalkene compound described in US. Patent Nos. 4,696,949 and 5,491,173 to Toivola et al., the disclosures of which are incorporated herein by reference. The parenteral and topical administration to mammalian subjects of formulations containing toremifene are described in U.S. Patent No. 5,571,534 to Jalonen et al. and in U.S. Patent No. 5,605,700 to DeGregorio et al., the disclosures of which are incorporated herein by reference.

U.S. Patent No. 5,595,985 to Labrie, the disclosure of which is incorporated herein by reference, also describe a method for treating benign prostatic hyperplasia using a combination of a 5α-reductase inhibitor and a compound that binds and blocks access to androgen receptors. One example of a compound that blocks androgen receptors is flutamide.

U.S. Patent Nos. 4,329,364 and 4,474,813 to Neri et al., the disclosures of which are incorporated herein by reference, describe pharmaceutical preparations comprising flutamide for delaying and/or preventing the onset of prostate carcinoma. The preparation can be in the form of a capsule, tablet, suppository, or elixir. Despite these developments, there is a continuing need for agents and methods effective for preventing prostate cancer.

Because of the high incidence and mortality of prostate cancer, it is imperative to develop chemoprevention strategies against this devastating disease. Understanding those factors that contribute to prostate carcinogenesis including the initiation, promotion, and progression of prostate cancer will provide molecular mechanistic clues as to appropriate points of intervention to prevent or halt the carcinogenic process. New innovative approaches are urgently needed at both the basic science and clinical levels to decrease the incidence of prostate cancer as well as to halt or cause the regression of latent prostate cancer. As the frequency of prostate cancer escalates dramatically at the same ages when men are confronted by other competing causes of mortality, simply slowing the progression of prostate adenocarcinoma may be both a more

suitable and cost effective health strategy. The present invention is directed to satisfying this need.

Further, as prostate intraepithelial neoplasia is in the direct causal pathway to prostate cancer and its presence specifically portends an increased risk of prostate cancer, men diagnosed with prostate intraepithelial neoplasia have dramatic changes in their quality of life. The only way to diagnose prostate intraepithelial neoplasia is by prostate biopsy. Once the diagnosis of prostate intraepithelial neoplasia is made, however, the standard of medical care is that the patient must be subjected to more frequent biopsies and physician visits. In addition, there is great patient and physician anxiety because the diagnosis of prostate cancer is imminent. Currently, there is no treatment available for patients who have prostate intraepithelial neoplasia.

SUMMARY OF THE INVENTION

This invention related to a method for preventing prostate carcinogenesis of a subject comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof; and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

This invention relates to method of suppressing or inhibiting latent prostate cancer of a subject comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof, and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

This invention relates to a method for reducing the risk of developing prostate cancer of a subject comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof, and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

This invention relates to a method for increasing the survival rate of a subject having prostate cancer comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof, and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

This invention relates to a method of treating a subject with prostate cancer comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof, and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

This invention relates to a method for reducing the amount of precancerous precursors of prostate adenocarcinoma lesions of a subject comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof, and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

In one embodiment the antiestrogen is a selective estogen receptor modulator (SERM), a triphenylethylene or a triphenyalkane. In one embodiment the precancerous precursor of prostate adenocarcinoma is prostate intraepithelial neoplasia (PIN). In one embodiment the precancerous precursor of prostate adenocarcinoma is high grade prostate intraepithelial neoplasia (PIN).

The present invention provides a safe and effective method for preventing prostate carcinogenesis , suppressing or inhibiting latent prostate cancer and is particularly useful for treating subjects having an elevated risk of developing prostate cancer, for example, those having benign prostatic hyperplasia, prostate intraepithelial neoplasia (PIN), or an abnormally high level of circulating prostate specific antibody (PSA), or who have a family history of prostate cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: A graph illustrating the chemopreventive effects of toremifene in the TRAMP model.

Figures 2A-2C: H&E sections illustrating ventral prostate cells in normal mice and prostate carcinoma in TRAMP mice included in the study.

Figure 3: Effect of Toremifene on ventral prostate development in the TRAMP mouse.

Figure 4: Effect of Toremifene on tumor occurrence in the TRAMP mice.

Figure 5: Effect of Toremifene on tumor development in the TRAMP model.

Figures 6A-6B: Comparison of placebo vs. Toremifene effects on tumor growth.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a: 1) method for preventing prostate carcinogenesis; 2) methods for suppressing or inhibiting prostate cancer; 3) methods for reducing the risk of developing prostate cancer; and 4) methods for increasing the survival rate of a subject; 5) methods of treating prostate cancer; 6) methods for regressing prostate intraepithelial neoplasia and methods for reducing the amount of high grade prostate intraepithelial neoplasia lesions by administrating the chemopreventive agents as provided herein.

In one embodiment the chemopreventive agent is an antiestrogen, its analog, derivative, isomer, and metabolite thereof. In another embodiment the chemopreventive agent is a tri-phenylalkaneor, its analog, derivative, isomer,

and metabolite thereof. In another embodiment the chemopreventive agent is a dihydronapthalene, its analog, derivative, isomer, and metabolite thereof. In another embodiment the chemopreventive agent is a benzothiopheneor its analog, derivative, isomer, and metabolite thereof. In another embodiment the chemopreventive agent is a selective estrogen receptor modulator (SERM) and its analog, derivative, isomer, and metabolite thereof. In another embodiment, the antiestrogen is a non DNA adduct forming antiestrogen and its analog, derivative, isomer, and metabolite thereof. In one embodiment the chemopreventive agent is tamoxifen. In another embodiment the chemopreventive agent is faslodex. In another embodiment the chemopreventive agent is raloxifene.

As demonstrated herein, tamoxifen, faslodex, and raloxifene, which are examples of antiestrogens is a prostate chemopreventive agent and prostate intraepithelial neoplasia agent.

Intermediate endpoint biomarkers are measurable biologic alterations in tissue that occur between the initiation of and the development of frank neoplasia. A biomarker is validated if the final endpoint, cancer incidence, were also reduced by the putative chemopreventive agent. Intermediate biomarkers in cancer may be classified into the following groups: histologic, proliferation, differentiation and biochemical markers. In any chemoprevention strategy, the availability of histologically recognizable and accepted precancerous lesions constitutes an important starting point. For the prostate, a histological marker is a precancerous precursor of prostatic adenocarcinoma of which prostatic intraepithelial neoplasia (PIN) is an example of. PIN appears as an abnormal proliferation within the prostatic ducts of premalignant foci of cellular dysplasia and carcinoma in situ without stromal invasion. PIN and histological prostate cancer are morphometrically and phenotypically similar. Thus, the development of high grade PIN represents an important step in the progression pathway whereby the normal prostate develops PIN, histological prostate cancer, invasive clinical prostate cancer, and metastases.

Prostate intraepithelial neoplasia has been shown to be a precancerous lesion, or precursor of prostatic adenocarcinoma. Prostate intraepithelial neoplasia is the abnormal proliferation within the prostatic ducts of premalignant foci of cellular dysplasia and carcinoma in situ without stromal invasion. prostate intraepithelial neoplasia is the most accurate and reliable marker of prostate carcinogenesis and may be used as an acceptable endpoint in prostate chemoprevention trials. Prostate intraepithelial neoplasia has a high predictive value as a marker for adenocarcinoma, and its identification warrants repeat biopsy for concurrent or subsequent invasive carcinoma. Most studies suggest that most patients with prostate intraepithelial neoplasia will develop carcinoma within 10 years. Interestingly, prostate intraepithelial neoplasia does not contribute to serum PSA, which is not surprising, since unlike prostate cancer, prostate intraepithelial neoplasia has not yet invaded the vasculature of the prostate to leak PSA into the blood stream. Thus, prostate intraepithelial neoplasia may precede even prostate cancer related serum PSA elevations.

In one embodiment, antiestrogens which act as prostate chemopreventive agents include but are not limited to: triphenylethylenes which include droloxifene, idoxifene, (2)-4-OH-tamoxifene; arzoxifene; chromans such as levomeloxifene, and centchroman; benzothiophenes such as raloxifene, and LY 353381; naphthalens such as CP336,156. In another embodiment, the chemopreventive agent includes phytoestrogens such as isoflavanoids including daidzein, genistein, yenoestrogens; coumestrol; zearalenone; daidzein; apigenin; waempferol; phloretin; biochanin A; naringenin; formononetin; ipriflavone; quercetin; and chrysin. In another embodiment, the chemopreventive agent includes. In another embodiment, the chemopreventive agent includes flavonoids; flavones, isoflavones, flavanones, and chalcones); coumestans; mycoestrogens; resorcyclic acid lactone; nafoxideneand equol, and lignan including enterodiol and enterolactone. In another embodiment, the chemopreventive agent includes the following compounds: ICI 164,384, ICI 182, 780; TAT-59, EM-652 (SCG 57068), EM-800 (SCH57050), EM-139, EM-651,

EM-776, and peptide antagonist of human estrogen receptors. In another embodiment, the chemopreventive agent includes the compounds and their analogs, derivatives, intermediates, isomers, metabolites which are disclosed in US Patent Nos: 4,696,949, 4,996,225, 5,491,173, which are herby incorporated by reference.

This invention provides the use of a composition and a pharmaceutical composition for a preventing prostate carcinogenesis; suppressing or inhibiting prostate cancer; reducing the risk of developing prostate cancer; increasing the survival rate of a subject; treating prostate cancer; regressing prostate intraepithelial neoplasia and reducing the amount of high grade prostate intraepithelial neoplasia lesions by administrating the chemopreventive agents as provided hereinabove and a carrier or diluent and/or their pharmaceutically acceptable carrier, diluents, salts, esters, or N-oxides, and mixtures thereof.

The present invention provides a safe and effective method for preventing carcinogenesis, suppressing or inhibiting latent prostate cancer and is particularly useful for treating subjects having an elevated risk of developing prostate cancer, for example, those having benign prostatic hyperplasia, prostate intraepithelial neoplasia (PIN), or an abnormally high level of circulating prostate specific antibody (PSA), or who have a family history of prostate cancer. In one embodiment the subject is a mammalian subject. In another embodiment the subject is a human subject.

The invention encompasses pure (Z)- and (E)- isomers of the compounds and mixtures thereof as well as pure (RR,SS)- and (RS,SR)-enantiomer couples and mixtures thereof.

The invention includes pharmaceutically acceptable salts of amino-substituted compounds with organic and inorganic acids, for example, citric acid and hydrochloric acid. The invention also includes N-oxides of the amino substituents of the compounds of formula (I). Pharmaceutically acceptable salts can also be

prepared from the phenolic compounds by treatment with inorganic bases, for example, sodium hydroxide. Also, esters of the phenolic compounds can be made with aliphatic and aromatic carboxylic acids, for example, acetic acid and benzoic acid esters.

As used herein, pharmaceutical composition means therapeutically effective amounts of the agent together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvant and/or carriers. A "therapeutically effective amount" as used herein refers to that amount which provides a therapeutic effect for a given condition and administration regimen. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (e.g., Tris-HCl., acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts). solubilizing agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance. Controlled or sustained release compositions include formulation in lipophilic depots (e.g., fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g., poloxamers or poloxamines). Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral. In one embodiment the pharmaceutical composition is administered parenterally, paracancerally, transmucosally, transdermally, intramuscularly, intravenously, intradermally,

subcutaneously, intraperitonealy, intraventricularly, intracranially and intratumorally. The dosage may be in the range of 5-80 mg/day. In another embodiment the dosage is in the range of 35-66 mg/day. In another embodiment the dosage is in the range of 40-60 mg/day. In another embodiment the dosage is in a range of 45-60 mg/day. In another embodiment the dosage is in the range of 15-25 mg/day. In another embodiment the dosage is in the range of 55-65 mg/day. In another embodiment the dosage is in a range of 45-60 mg/day. The dosage may be 60 mg/day. The dosage may be 20 mg/day. The dosage may be 45 mg/day. tablet for oral administration that contains 88.5 mg of the chemopreventive agent, which is equivalent to 60 mg of toremifene

Further, as used herein pharmaceutically acceptable carrier are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, collating agents, inert gases and the like.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood et al., *Immunology, Second Ed.*, 1984, Benjamin/Cummings: Menlo Park, California, p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail

to elicit a humoral or cellular immune response. Adjuvant include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvant such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*. Preferably, the adjuvant is pharmaceutically acceptable.

Controlled or sustained release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral. Compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline are known to exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified compounds (Abuchowski et al., 1981; Newmark et al., 1982; and Katre et al., 1987). Such modifications may also increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. As a result, the desired *in vivo* biological activity may be achieved by the administration of such polymer-compound abducts less frequently or in lower doses than with the unmodified compound.

In yet another embodiment, the pharmaceutical composition can be delivered in a controlled release system. For example, the agent may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch,

liposomes, or other modes of administration. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989). In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984). Preferably, a controlled release device is introduced into a subject in proximity of the site of inappropriate immune activation or a tumor. Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990).

The method of the present invention for preventing prostate carcinogenesis involves administering to a mammalian subject a pharmaceutical preparation comprising chemopreventive agent or a metabolite or salt thereof. The pharmaceutical preparation can comprise the chemopreventive agent alone, or can further include a pharmaceutically acceptable carrier, and can be in solid or liquid form such as tablets, powders, capsules, pellets, solutions, suspensions, elixirs, emulsions, gels, creams, or suppositories, including rectal and urethral suppositories. Pharmaceutically acceptable carriers include gums, starches, sugars, cellulosic materials, and mixtures thereof. The pharmaceutical preparation containing the chemopreventive agent can be administered to a subject by, for example, subcutaneous implantation of a pellet; in a further embodiment, the pellet provides for controlled release of chemopreventive agent over a period of time. The preparation can also be administered by intravenous, intraarterial, or intramuscular injection of a liquid preparation, oral administration of a liquid or solid preparation, or by topical application. Administration can also be accomplished by use of a rectal suppository or a urethral suppository. The pharmaceutical preparation can also be a parenteral formulation; in one embodiment, the formulation comprises a liposome that includes a complex of a chemopreventive agent such as, for example, toremifene and a cyclodextrir compound, as described in the previously cited U.S. Patent No. 5,571,534 to

The pharmaceutical preparations of the invention can be prepared by known dissolving, mixing, granulating, or tablet-forming processes. For oral administration, the chemopreventive agents or their physiologically tolerated derivatives such as salts, esters, N-oxides, and the like are mixed with additives customary for this purpose, such as vehicles, stabilizers, or inert diluents, and converted by customary methods into a suitable form for administration, such as tablets, coated tablets, hard or soft gelatin capsules, aqueous, alcoholic or oily solutions. Examples of suitable inert vehicles are conventional tablet bases such as lactose, sucrose, or cornstarch in combination with binders like acacia, cornstarch, gelatin, or with disintegrating agents such as cornstarch, potato starch, alginic acid, or with a lubricant like stearic acid or magnesium stearate. Examples of suitable oily vehicles or solvents are vegetable or animal oils such as sunflower oil or fish-liver oil. Preparations can be effected both as dry and as wet granules. For parenteral administration (subcutaneous, intravenous, intraarterial, or intramuscular injection), the chemopreventive agents or their physiologically tolerated derivatives such as salts, esters, N-oxides, and the like are converted into a solution, suspension, or emulsion, if desired with the substances customary and suitable for this purpose, for example, solubilizers or other auxiliaries. Examples are: sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solutions, and glycols such as propylene glycols or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions.

The preparation of pharmaceutical compositions which contain an active component is well understood in the art. Typically, such compositions are prepared as an aerosol of the polypeptide delivered to the nasopharynx or as injectables, either as liquid solutions or suspensions, however, solid forms

suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

An active component can be formulated into the composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

For topical administration to body surfaces using, for example, creams, gels, drops, and the like, the chemopreventive agents or their physiologically tolerated derivatives such as salts, esters, N-oxides, and the like are prepared and applied as solutions, suspensions, or emulsions in a physiologically acceptable diluent with or without a pharmaceutical carrier.

In another embodiment, the active compound can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid).

The pharmaceutical compositions of the present invention are particularly useful for treating a subject having an elevated risk of developing prostate cancer. High-risk subjects include, for example, those having benign prostatic hyperplasia, prostatic intraepithelial neoplasia (PIN), or an abnormally high level of circulating prostate specific antibody (PSA), or who have a family history of prostate cancer.

Further, the prostate chemopreventive agent may be administered in combination with other cytokines or growth factors include but are not limited to: IFN γ or α, IFN-β; interleukin (IL) 1, IL-2, IL-4, IL-6, IL-7, IL-12, tumor necrosis factor (TNF) α, TNF-β, granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage CSF (GM-CSF); accessory molecules, including members of the integrin superfamily and members of the Ig superfamily such as, but not limited to, LFA-1, LFA-3, CD22, and B7-1, B7-2, and ICAM-1 T cell costimulatory molecules.

The chemopreventive agent may precede or follow a DNA damaging agent treatment by intervals ranging from minutes to weeks. Protocols and methods are known to those skilled in the art. DNA damaging agents or factors are known to those skilled in the art and means any chemical compound or treatment method that induces DNA damage when applied to a cell. Such agents and factors include radiation and waves that induce DNA damage such as, gamma -irradiation, X-rays, UV-irradiation, microwaves, electronic emissions, and the like. A variety of chemical compounds, also described as "chemotherapeutic agents", function to induce DNA damage, all of which are intended to be of use in the combined treatment methods disclosed herein. Chemotherapeutic agents contemplated to be of use, include, e.g., adriamycin, 5-fluorouracil (5FU), etoposide (VP-16), camptothecin, actinomycin-D, mitomycin C, cisplatin (CDDP) and even hydrogen peroxide. The invention also encompasses the use of a combination of one or more DNA damaging agents, whether radiation-based or actual compounds, such as the use of X-rays with cisplatin or the use of cisplatin with etoposide.

In another embodiment one may irradiate the localized tumor site with DNA damaging radiation such as X-rays, UV-light, gamma -rays or even microwaves. Alternatively, the tumor cells may be contacted with the DNA damaging agent by administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a DNA damaging compound such as, adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, or more preferably, cisplatin. Agents that damage DNA also include compounds that interfere with DNA replication, mitosis and chromosomal segregation. Such chemotherapeutic compounds include adriamycin, also known as doxorubicin, etoposide, verapamil, podophyllotoxin, and the like.

Other factors that cause DNA damage and have been used extensively include what are commonly known as gamma -rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated such as microwaves and UV-irradiation. It is most likely that all of these factors effect a broad range of damage DNA, on the precursors of DNA, the replication and repair of DNA, and the assembly and maintenance of chromosomes.

As can be readily appreciated by one of ordinary skill in the art, the methods and pharmaceutical compositions of the present invention are particularly suited to administration to a mammal, preferable a human subject.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXPERIMENTAL DETAILS SECTION

EXAMPLE I: Transgenic Adenocarcinoma Mouse Prostate

The study of prostate cancer chemoprevention has been hindered by the lack of appropriate animal models. The recent development of the transgenic adenocarcinoma mouse prostate (TRAMP) model enables the study of chemoprevention. In the TRAMP model, which is described in Greenberg et al., AProstate cancer in a transgenic mouse, Proc. Nat1 Acad. Sci. USA, 1995, Vol. 92, pages 3439-3443, the PB-SV40 large T antigen (PB-Tag) transgene is expressed specifically in the epithelial cells of the murine prostate. As a result, this model has several advantages over currently existing models: 1) mice develop progressive forms of prostatic epithelial hyperplasia as early as 10 weeks and invasive adenocarcinoma around 18 weeks of age; 2) the metastatic spread of prostate cancer pattern mimics human prostate cancer with the common sites of metastases being lymph node, lung, kidney, adrenal gland, and bone; 3) the development as well as the progression of prostate cancer can be followed within a relatively short period of l0-30 weeks; 4) the tumors arise with 100% frequency; and 5) the animals may be screened for the presence of the prostate cancer transgene prior to the onset of clinical prostate cancer to directly test treatment with chemopreventive agents that may alter prostate carcinogenesis.

The TRAMP transgenic mouse model is an excellent *in vivo* model to determine the mechanisms of initiation and promotion of prostate *cancer* and to test the effectiveness of potential chemopreventive agents. These mice progressively develop prostatic epithelial hyperplasia, PIN, and then prostate cancer within a short period (<17 weeks).

Chemopreventive treatment of hybrid TRAMP mice is initiated 30 days postnatally, using chemopreventive agents at a level of about 0.5-50 mg/kg of

subject weight/day, preferably about 6-30 mg/kg of subject weight/day. The chemopreventive agents are conveniently processed into 21-day and 90-day pellets (prepared by Innovative Research of America, Sarasota, FL) and delivered as subcutaneous implants. Control animals receive placebo implants. In each drug treatment group, animals are sacrificed at 5,7, 10, 15,20,25,30,40, and 50 weeks of age until the development of a palpable tumor. Blood is collected and pooled per treatment time point to evaluate changes in serum testosterone and estradiol. Prostatic tissues are harvested for morphometric, histologic, and molecular studies.

The following test procedures are employed:

1) Prostate wholemount analysis is serially performed to detect changes in prostate ductal morphology over time with and without treatment; examples are shown in Fig. 2. Tissue sections are evaluated histologically by H&E and Masson-trichome standard staining. The emergence of PIN is assessed and graded (I-mild to III-severe).

2) Serum estradiol and total testosterone levels are measured (RIA) for each age interval to assess any changes in these hormones as a result of chemopreventive agents.

EXAMPLE 2: Immunohistochemistry Data Analysis

Microscopy images of each tissue section are evaluated by using computer-assisted (Mac 9500-I 32 computer and monitor) image quantitation (NIH-Image 1.6 PPC) using Kodak DCS 460 camera on Nikon Microphot-FX microscope and quantitated by using a color-assisted quantitative system image analysis (IPLab Spectrum 3.1, Scanalytics, Inc., VA) that discriminates color differences of stained tissue sections. Thresholds are set to identify various tissue components of the prostate. The area pixel densities corresponding to each of these tissue components are calculated for each full screen of the color monitor. A total of 5 screens per prostate section are averaged. Immunohistochemical images can be digitalized and quantitated to enable

statistical evaluation by determination of sample correlation coefficients and probability (2-tailed).

EXAMPLE 3: Study of Chemopreventive Activity

A study was undertaken to test the efficacy of chemopreventive agents in TRAMP transgenic animals (PBTag X FVBwt)(provided by Dr. Norman Greenberg, Baylor College of Medicine, TX). These mice showed preliminary signs of cancer as early as 10 weeks. The TRAMP transgenic male litters were screened for the Large T ag transgene, and the positive males were used in the study. The antiestrogen toremifene, which was to be tested for its possible chemopreventive effects, was incorporated in customized pellets (Innovative Research of America, Sarasota, FL), and chemopreventive treatment of mice was initiated postnatally at 30 days (average mouse weight 14g). Four groups of l0-12 animals each received subcutaneous implantations of 90 day-release toremifene-containing pellets. The diffusible drug dosage, adjusted for growth related changes in weight, was designed to deliver either a low dose (6mg/kg) or a high dose (30mg/kg) of toremifene. Control animals (n=lO) received placebo implants. The efficacy of the treatment was measured by the absence of palpable tumor formation. The murine prostate tumors were harvested and evaluated by molecular and histological techniques.

Using the TRAMP transgenic model of prostate cancer, in which every animal that inherits the prostate cancer gene develops prostate cancer, it was demonstrated that toremifene both increases the latency and decreases the incidence of prostate cancer.

As shown in Figure 1 the effects of low and high dose toremifene were both effective. Tumor formation in the TRAMP mouse ventral prostate was noted at week 17 for the placebo group (n=lO), at week 19 for the high dose toremifene-treated group(n=12), and at week 28 for the low dose toremifene-treated group (n=12). Thus, 5 treatment by toremifene substantially

increased the latency period by up to 11 weeks for the development of cancer in the ventral prostate of TRAMP mice.

Since the toremifene-treated animals did not reach the 50% tumor development point during the period of the study, the time in which 25% of the animals had tumors was compared among groups. Tumors were palpable in 25% of 10 the animals by week 23 in the placebo group and by 30-31 weeks in the high and low toremifene groups, a delay of 7-8 weeks. Both low toremifene and high toremifene vs placebo were significant by log rank and Wilcoxon statistical analysis, as shown in Table 1 below.

Table 1 - Statistical Analysis

	Log-Rank P	Wilcoxon p
Low toremifene vs placebo	0.0003 *	0.0004*
High toremifene vs placebo	0.0017*	0.0071*

*significance P<0.05

At week 33, a point when all of the control animals had developed tumors, 72% of the low dose and 60% of the high dose toremifene-treated animals were still tumor-free. Thus, toremifene treatment at both low and high dosages resulted in a greatly decreased incidence of tumors in the ventral prostate of TRAMP mice. These data demonstrated that the incidence of prostate cancer was significantly decreased and increased the latency period.

As already discussed, administering toremifene produces a substantial chemopreventive effect against tumors in the ventral prostate of TRAMP mice. This result is encouraging for a similar beneficial effect on human subjects, whose prostate does include a segment corresponding to the ventral prostate of rodents.

Example 4: Histological Examination of Prostate Tissue

Tumors from the placebo and high toremifene- treated groups taken at the time of palpation were evaluated histologically. Figure 2A is an H&E section of the ventral prostate of a 17-week-old normal adult mouse. Figure 2B, a section of the ventral prostate of a placebo-treated 16-week-old TRAMP mouse, shows that, unlike the normal prostate structure depicted in Figure 2A, the TRAMP mouse ventral prostate is characterized by sheets of undifferentiated, anaplastic cells with a high mitotic index. In contrast, as shown in Figure 2C, the prostate of a toremifene-treated 30-week-old TRAMP mouse retains much of the normal glandular architecture and ha tumors with a more differentiated structure, the mitotic index being much lower than that for the placebo-treated animal. These results indicate that toremifene, even at low dosage, is able to suppress prostate carcinogenesis in the TRAMP model.

Western blot analysis: Prostate tissues (dorsolateral and ventral lobes) were harvested at 10 weeks of age, snap-frozen in liquid N_2 and stored at –80°C. Tissue lysates were prepared using RIPA buffer (150 mM NaCl, 1% NP40, 0.5% deoxycholate, 0.1% SDS and 50 mM Tris, pH 7.5) containing a cocktail of protease inhibitors (Pefabloc, aprotinin, bestatin, leupeptin and pepstain) and the phosphatase inhibitor Na_3VO_4 (10mM). The homogenate was centrifuged at 14,000x g at 4°C for 10 minutes and lysates were stored at –80°C.

Protein concentrations were determined using the Bradford protein assay (Bio-Rad). Tissue lysates were loaded onto 7.5% polyacrylamide gels, proteins (40µg/lane) separated by SDS-PAGE, and electrophoretically transferred to nitrocellulose membranes (0.2 µm, Bio-Rad, Hercules, CA) in transfer buffer (192 mM glycine, 25 mM Tris-HCl and 20% methanol). TRAMP prostate tumor tissue was used as positive control. Chemiluminescent Cruz Markers (Santa Cruz Biotechnology, Santa Cruz, CA) were used as molecular weight standards. Blots were blocked overnight at 4°C in BLOTTO (6% non-fat dry milk in 1X TBS) and incubated with the large T-antigen primary antibody (Pab 101 mouse monoclonal, 1:200, Santa Cruz Biotechnology) for 2 hours at room temperature.

The blots were washed (3x) with TTBS (0.05% Tween 20, 50mM Tris-Hcl, 200mM NaCl) and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000) for 1 hour at 25°C. Immunoreactive proteins were visualized on autoradiography film using the enhanced chemiluminescence (ECL) system (APB, Piscataway, NJ). Actin protein expression was used to normalize Tag results. For this purpose, the above membrane was submerged in stripping buffer (100 mM 2-mercaptoethanol, 2%SDS, 62.5mM Tris-Hcl pH 6.7) and incubated at 50°C for 30 minutes with occasional agitation. After blocking the membrane was reprobed with actin primary antibody (1:2500, Chemicon, Temecula, CA) followed by (HRP)-conjugated secondary antibody (1:10000). Following ECL detection, band intensities were quantitated using Adobe Photoshop 5.0 Acquisition and ImageQuant Analysis (Molecular Dynamics) systems.

EXAMPLE 5 : Use of Chemopreventive Efficacy of Toremifene Against Prostate Cancer in the TRAMP Mouse Model

This experiment confirms and demonstrates the chemopreventive efficacy of toremifene. This present study focuses on the histological and molecular changes associated with development of prostate tumor in control animals and the mechanism of toremifene chemopreventive action with TRAMP animals which are bred, screened and treated with sustained-release drug pellets. At predetermined times, groups of 5 animals were sacrificed and their prostates were removed for analysis. The prostate glands were evaluated for the presence of tumor by histology, wholemount dissections, and large T antigen immunohistochemistry. To date, the Placebo and the Toremifene treatments have been completed for the 7, 10, 15 and 20 week time-points and the results are described below.

Results: Prostatic wholemounts for 7,10,15, and 20 weeks for the various groups have been completed. Wholemount analysis revealed that placebo treated mice developed prostate tumors by 15-20 weeks of age similar to the previous pilot

study. Moreover, the Toremifene treated animals had a delay in the occurrence of prostate cancer up to 20 weeks (Figure 3). By 20 weeks, there is a striking delay in tumor occurrence in the Toremifene treated group up to 35 weeks Figure 4). These data confirm that even with a more sensitive assessment of tumorigenicity, Toremifene exhibited chemopreventive activity. For histological evaluation, tissue samples were fixed, processed and paraffin embedded. Sections (5pM thick) were cut and stained by routine H&E method. Toremifene inhibited the ductal development and tissue differentiation (compare the 17 weeks TRAMP mouse prostate tumor vs. wildtype (Figure 4) ; b) Toremifene treated prostate histology vs. Placebo at 15 weeks (Figure 5) Qualitatively, immunohistochemistry of Placebo and Toremifene treated tissues showed presence of T-antigen in the ventral prostate. Thus, the chemopreventive activity seen by Toremifene does not appear to be by suppression of the probasin promoter in the TRAMP model.

Conclusions: The ability of Toremifene to prevent the occurrence of prostate cancer in the TRAMP model has been confirmed utilizing more sensitive techniques to assess tumor formation. The mechanism of Toremifene=s chemopreventive effects does not appear to be through loss of the transgene for the Large T- antigen protein.

EXAMPLE 6 : Toremifene Induces Regression of Established Human Prostate Cancer Tumors in the Nude Mouse Model

Prostate cancer currently remains the most commonly diagnosed cancer in American males. However, questions remain about the etiology and treatment of this disease especially is advanced forms. Hormone therapy remains the standard method of treatment for recurrent and advanced prostate cancer despite the common development of hormone refractory disease. Therefore, new approaches for the prevention and treatment of prostate cancer are needed to accommodate the increasing number of men diagnosed with this disease. The experiments and results below demonstrate that toremifene suppresses

hormone sensitive LNCaP tumor growth in athymic nude mice.

Materials and Methods: One million LNCaP cells in Matrigel were subcutaneously injected into each flank of athymic nude mice. A total 40 mice were injected. After approximately 3-4 weeks, visible tumors developed. After recording the tumor size in two dimensions, the mice were divided into placebo and treatment groups based on equivalent tumor burden. A single pellet (placebo versus toremifene 35 mg) was subcutaneously implanted between the scapulae of each mouse. Weekly measurements of the tumor size were recorded. Tumor volume was calculated (tumor volume = 0.5 (L + W) x L x W x 0.5236, where L = tumor length and W = width). The tumor volume at the time of pellet implantation served as the point of reference for future comparison of that tumor=s size variation. The weekly variations of each tumor volume were recorded as percent differentiation from the original measurement at pellet implantation.

Results: Of the 78 tumor injection sites, 55 (70%) resulted in tumors of adequate volume for evaluation. A total of 50 tumors (24 placebo and 26 chemopreventive agent, toremifene, treated animals) were available for evaluation. Mean tumor volumes at the time of pellet implantation were similar for the chemopreventive agent, toremifene and placebo groups (1.90 mm^3 and 1.72 mm^3, respectively). Mean tumor volume decreased to 1.68 mm^3 in the chemopreventive agent, toremifene group (-0.22 mm^3), while mean tumor volume increased to 2.33 mm^3 in the placebo group (+0.61 mm^3). Mean serum PSA level was higher in the placebo group (3.80 mg/ml) than in the chemopreventive agent, toremifene group (2.80 ng/ml), but this was not statistically significant (p=0.755). Total testosterone serum levels were 2.18 ng/ml for the placebo group (n=17) and 2.96 ng/ml for the chemopreventive agent, toremifene group (n=19).

Two mice died soon after pellet implantation due to mortal wounds from other mice. One mouse treated with toremifene was excluded from the study due to excessive tumor hemorrhage and hematoma development. All mice developed

visible tumors unilaterally or bilaterally. Each tumor was followed independently for the duration of the study. Twenty-four tumors were treated with placebo and 28 tumors were treated with toremifene. The results are shown in Table 2and Figure 6A and 6B.

Table 2

PLACEBO GROUP

Week	N=	% Change in volume relative to day 0 of treatment
3	11	9.44
4	8	115.27
5	8	271.71
6	8	600.88

TOREMIFENE

Week	N=	% Change in volume relative to day 0 of treatment
3	11	-34.58
4	7	-61.01
5	7	-74.51
6	5	-61.72

The follow-up interval will be extended on the currently reported population and data on additional animals are presently being collected.

Conclusion: The chemopreventive agent, toremifene, inhibits and induces regression of established LNCaP tumors. Although the mechanism by which toremifene exerts this effect is unknown, the ability to produce these effects supports the use of Toremifene as a treatment for prostate cancer and to prevent the recurrence of prostate cancer in high risk patients with established prostate cancer micrometastases.

Example 7: The Role of Antiestrogens: Tamoxifen citrate and Raloxifene (SERMs) and Faslodex (pure antiestrogen ICI 182,780) in the prevention of Prostate Cancer

Experimental design: Chemopreventive treatment of mice are initiated post-natal at 30 days. Three groups of 50 hybrid TRAMP male mice each are treated with either Tamoxifen citrate, or Raloxifene (SERMs) or Faslodex (pure antiestrogen ICI 182,780). The drugs is obtained as customized sustained-release pellets (Innovative Research of America, Sarasota, FL) and delivered as subcutaneous implants (see preliminary data). Control animals are receive placebo implant with no pharmacological activity. Animals (n=10) are sacrificed at periodic intervals, 10, 15, 20, 25 and 30 weeks age and the efficacy of the treatment leading to either absence of tumor formation or reduction in tumor size, if present, are assessed by comparison with placebo control animals. Blood is collected to evaluate changes in serum androgens and estrogens with each treatment. Prostatic tissues is saved for: a) morphometric studies; b) for histologic studies the tissue will be fixed in 10% buffered formalin, processed and paraffin embedded; c) for molecular studies the tissues is frozen in liquid nitrogen and stored at –70°C. Necropsies and survival data is also recorded.

The results of the experiment reveal the relative chempreventive efficacy of the various antiestrogens in the delay or prevention of prostate cancer in the TRAMP model. The morphological studies indicate the gross changes, if any, in the development of the prostate size and ductal pattern as a result of each treatment. Paraffinized tissue sections are stained using standard H&E techniques for histological changes such as PIN that will be assessed to monitor the appearance of precancerous lesions as a precuror of prostatic adencarcinoma. Serum estradiol and total testosterone levels are measured for each age interval to assess any changes in these hormones, and whether or not they correlate to changes

in PIN. The peptide growth factor levels of TGF, TGF 1, TGF 3, and bFGF is quantitative in prostate samples taken at each interval. Corresponding peptide growth factor receptors is also assessed for EGFR and TGF RI and RII.

Table3:
The effects of Selective Estrogen Receptor Modulators (SERMs) on the prevention of prostate cancer in the Tramp model

SERM	Dose	20 wks # tumors	20 wks (% tumors)
Placebo	-	5/5	100%
Toremifene	20 mg/kg/d	1/7	14.2%
Tamoxifen	20 mg/kg/d	2/9	22%
Raloxifene	20 mg/kg/d	3/10	30%
* Faslodex (ICI 128,780)	* 10 mg/kg/d	8/11	72%

Animals were sacrificed at 20 weeks and prostate glands were evaluated by wholemount analysis and histologically.

*Faslodex is a pure antiestrogen and its relative potency is 2x that of the other SERMs, therefore 10mg/kg/d of Faslodex=20mg/kg/d of SERM.

Example 8: Toremifene causes regression of HGPIN in a Phase IIa prostate cancer chemoprevention human clinical trial

The chemopreventive effects of an antiestrogen, toremifene against prostate cancer have been reproducibly demonstrated herein in a well-established animal model of spontaneous human prostate cancer. This represents the first compound to demonstrate chemopreventive activity against prostate cancer. Moreover, High grade PIN (HGPIN) has been established and time tested as a precursor lesion for human prostate cancer also known as latent prostate cancer.

Consequently, PIN is used as an intermediate endpoint, or surrogate

endpoint for human prostate cancer. In fact, the NCI has now recommended that PIN should be used as an intermediate endpoint, or surrogate endpoint for human prostate cancer.

A Phase IIa,open labeled non randomized single center study with 21 human subjects was conducted. In this protocol, patients with biopsy proven PIN and who do not have prostate cancer are treated with 60mg of the chemopreventive agent, toremifene, daily for 4 months. After 4 months, patients are rebiopsied (8 biopsies) and PIN status is reassessed. Twenty-one patients entered the study and sixteen patients have completed the study. The summary of pathologic findings of the prostate biopsies of these 16 patients showed that 12 patients had regression of PIN to benign or atrophic prostate tissue; thus, 12 out of 16 (75%) patients had a complete response. Of the remaining 4 patients, 3 patients had prostate cancer but the amount of PIN was reduced, and 1 patient had stable disease, but the PIN epithelium demonstrated atrophic and degenerative changes.

The pathological evaluation revealed complete resolution of PIN with atrophic changes in the prostatic epithelum. The patient experienced no acute or chronic toxicites while taking Toremifene. The serum PSA, serum free testosterone, serum total testosterone, and serum estradiol remained in the normal ranges. Quality of life was unchanged including no affect on potency and libido. Therefore, these results demonstrate a prostate chemopreventive role for the antiestrogen toremifene.

The results demonstrate that the chemopreventive agents such as toremifene reduces PIN which thus directly translates to a decrease in the incidence and a prolongation of the latency of prostate cancer and preventing prostate carcinogenesis. Lastly, the chemopreventive agent, toremifene has been found to significantly induce TGF? synthesis in human stromal fibroblast cells.

WHAT IS CLAIMED IS:

1. A method for preventing prostate carcinogenesis of a subject comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof; and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

2. A method of suppressing or inhibiting latent prostate cancer of a subject comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof, and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

3. A method for reducing the risk of developing prostate cancer of a subject comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof, and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

4. A method for increasing the survival rate of a subject having prostate cancer comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof, and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

5. A method of treating a subject with prostate cancer comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof, and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

6. A method for reducing the amount of precancerous precursors of prostate adenocarcinoma lesions of a subject comprising: administering to a mammalian subject, a pharmaceutical preparation comprising an anti-estrogen, or its analog, derivative, isomer, and metabolite thereof, and their pharmaceutically acceptable salts, esters, or N-oxides, and mixtures thereof.

7. The method of claims 1-8, wherein the subject has precancerous precursors of prostate adenocarcinoma and does not have prostate cancer.

8. The method of claim 7, wherein the precancerous precursors of prostate adenocarcinoma is prostate intraepithelial neoplasia (PIN).

9. The method of claims 1-8, wherein the antiestrogen is a selective estogen receptor modulator (SERM), a triphenylethylene or a triphenyalkane.

10. The method according to claim 1-6 wherein said pharmaceutical preparation further comprises a pharmaceutically acceptable carrier.

11. The method according to claim 10, wherein said carrier is selected from the group consisting of a gum, a starch, a sugar, a cellulosic material, and mixtures thereof.

12. The method according to claim 10, wherein said administering comprises: subcutaneously implanting in said subject a pellet containing said pharmaceutical preparation.

13. The method according to claim 12, wherein said pellet provides for controlled release of said pharmaceutical preparation

over a period of time.

14. The method according to claim 10, wherein said administering intravenously, intraarterially, or intramuscularly injecting in said subject said pharmaceutical preparation in liquid form.

15. The method according to claim 10, wherein said administering orally administering to said subject a liquid or solid preparation containing said pharmaceutical preparation.

16. The method according to claim 10, wherein said administering topically applying to skin surface of said subject said pharmaceutical preparation.

17. The method according to claim 10, wherein said pharmaceutical preparation is selected from the group consisting of a pellet, a tablet, a capsule, a solution, a suspension, an emulsion, an elixir, a gel, a cream, and a suppository.

18. The method according to claim 17, wherein said suppository is a rectal suppository or a urethral suppository.

19. The method according to claim 10, wherein said pharmaceutical preparation is a parenteral formulation.

20. The method according to claim 19, wherein said parenteral formulation comprises a liposome comprising a complex of said chemopreventive agent and a cyclodextrin compound.

21. The method according to claim 10, wherein said administering is carried out at a dosage of about 0.5 mg/kg of subject weight/day to about 80 mg/kg of subject weight/day of said

chemopreventive agent.

22. The method according to claim 10, wherein said administering is carried out at a dosage of about 10 mg/kg of subject weight/day to about 60 mg/kg of subject weight/day of said chemopreventive agent.

23. The method according to claim 10, wherein said administering is carried out at about 20 mg/kg of subject weight/day to about 60 mg/kg of subject weight/day of said chemopreventive agent.

24. The method according to claim 10, wherein said administering is carried out at about 60 mg/kg of subject weight/day of said chemopreventive agent.

25. The method according to claim 10, wherein said administering is carried out at about 20 mg/kg of subject weight/day of said chemopreventive agent.

ABSTRACT OF THE INVENTION

This invention relates to the chemoprevention of prostate cancer and, more particularly, to a method of suppressing or inhibiting latent prostate cancer comprising administering to a mammalian subject a chemopreventive agent and analogs and metabolites thereof. The chemopreventive agent prevents, prevents recurrence of, suppresses or inhibit prostate carcinogenesis; and treats prostate cancer.

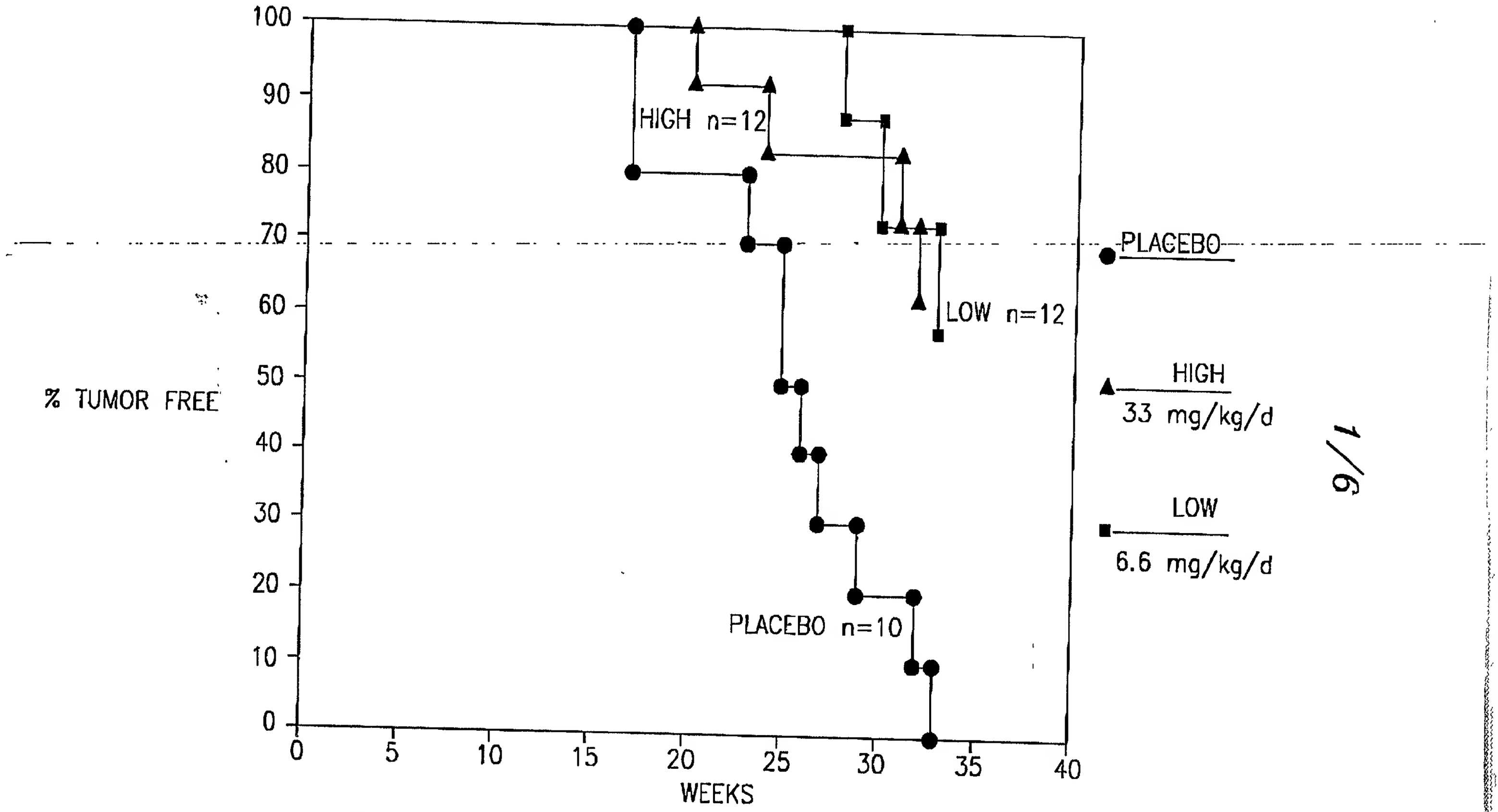
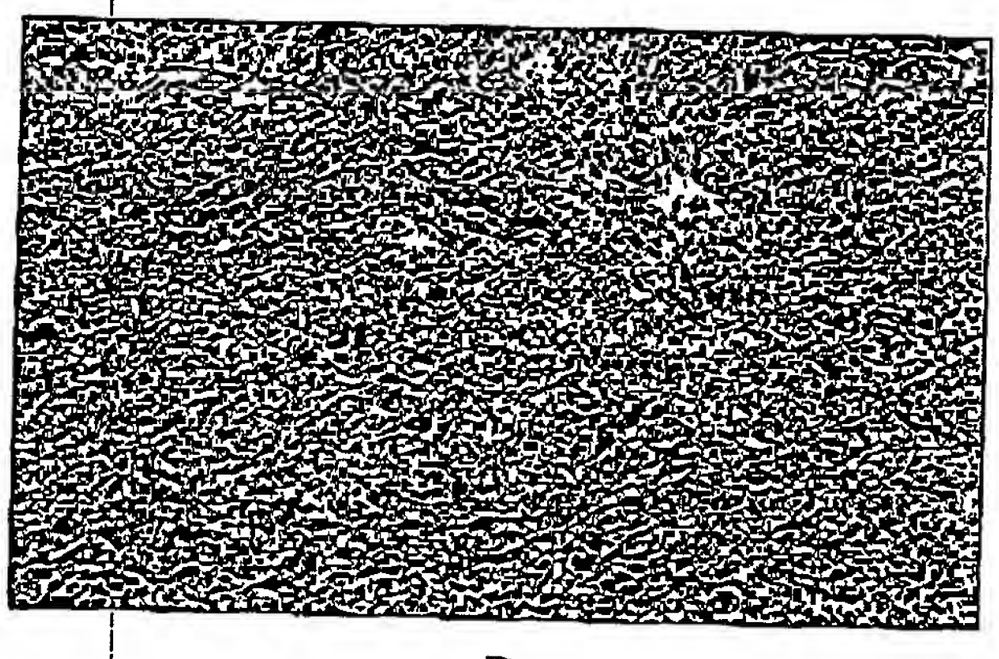

100
90
80
70
60
50
40
30
20
10
0
% TUMOR FREE
HIGH n=12
LOW n=12
PLACEBO n=10
PLACEBO
HIGH
33 mg/kg/d
LOW
6.6 mg/kg/d
0
5
10
15
20
25
30
35
40
WEEKS

FIG.1

A.



B.

C.

FIG.2

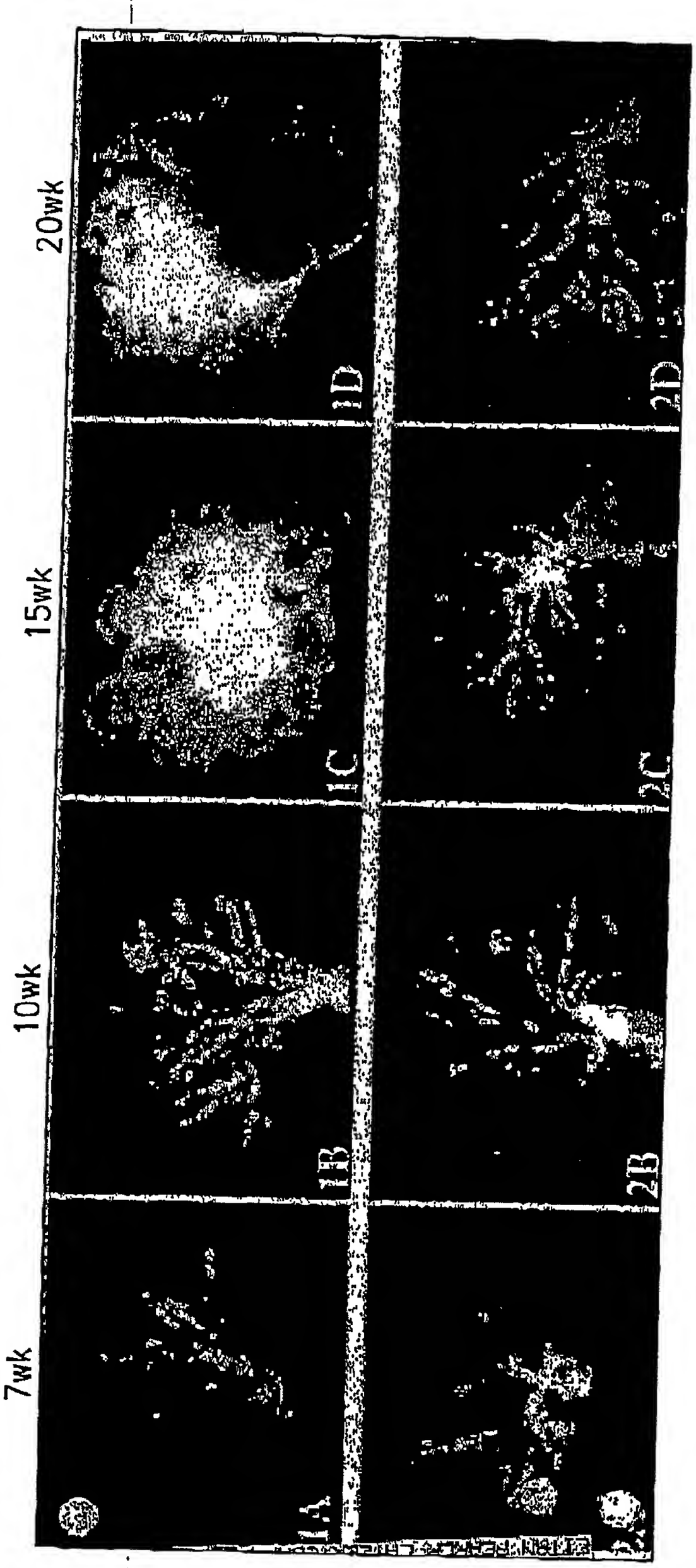


FIG.3

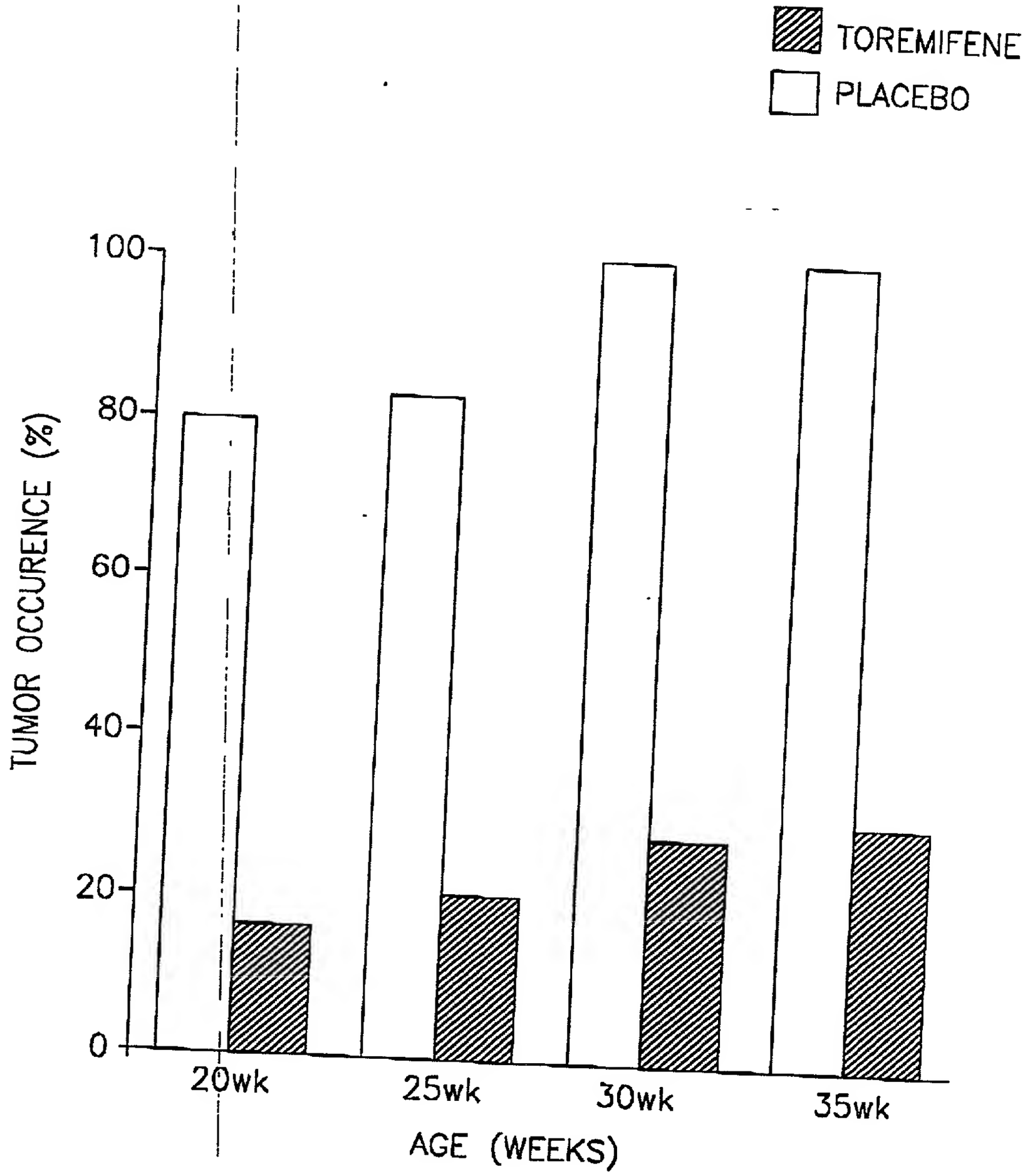

TOREMIFENE
PLACEBO
TUMOR OCCURENCE (%)
100
80
60
40
20
0
20wk
25wk
30wk
35wk
AGE (WEEKS)

FIG.4

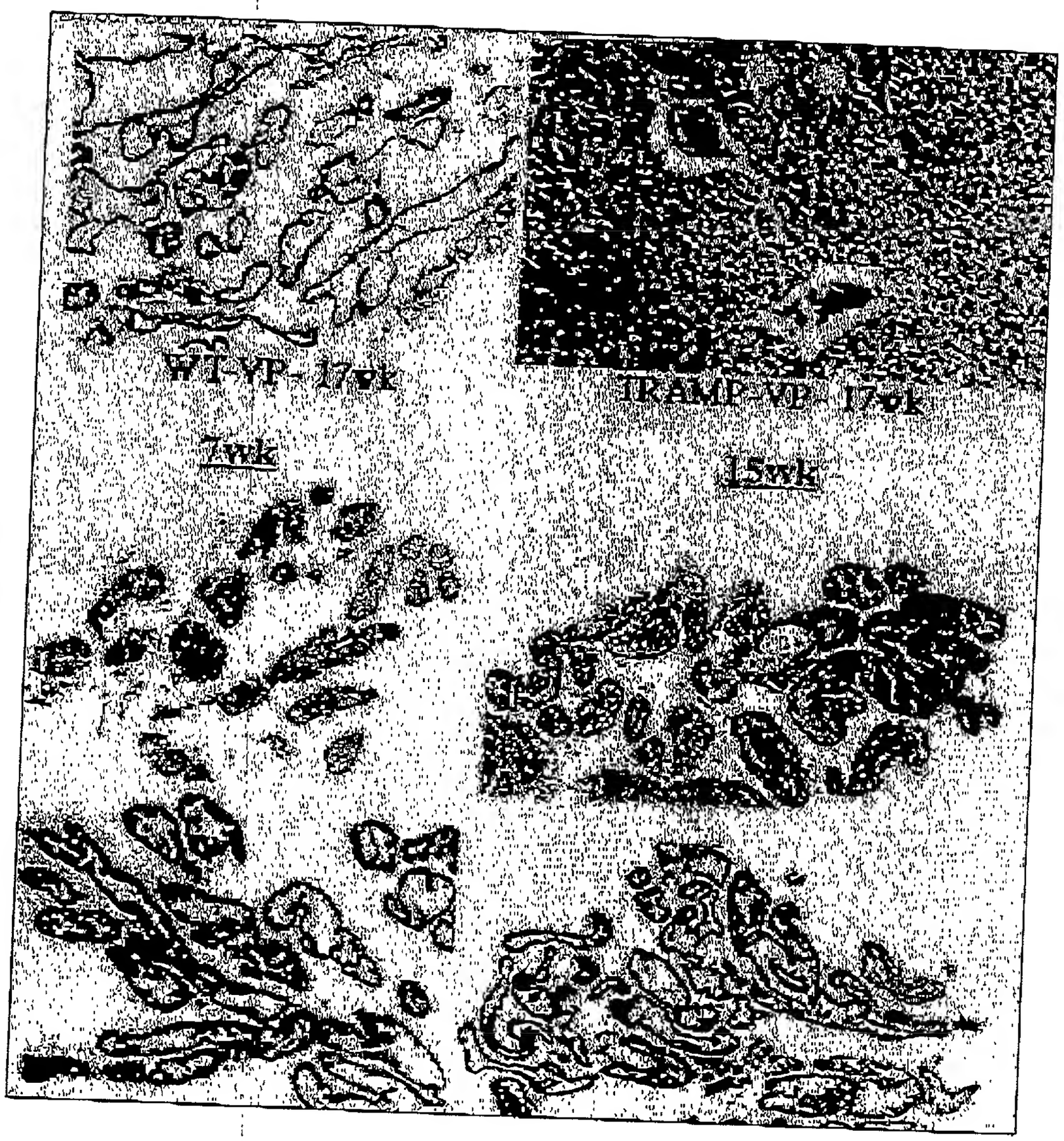
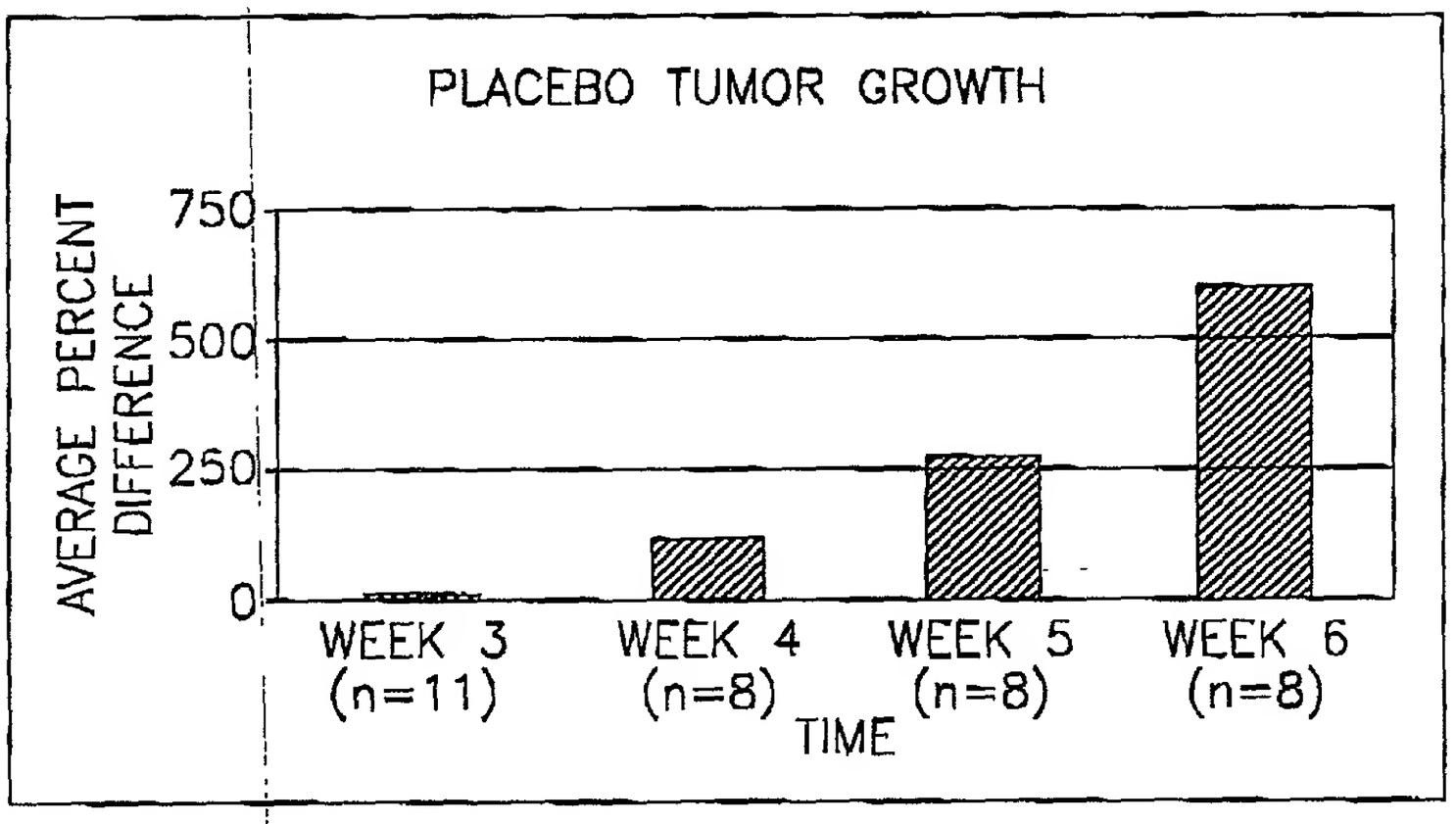
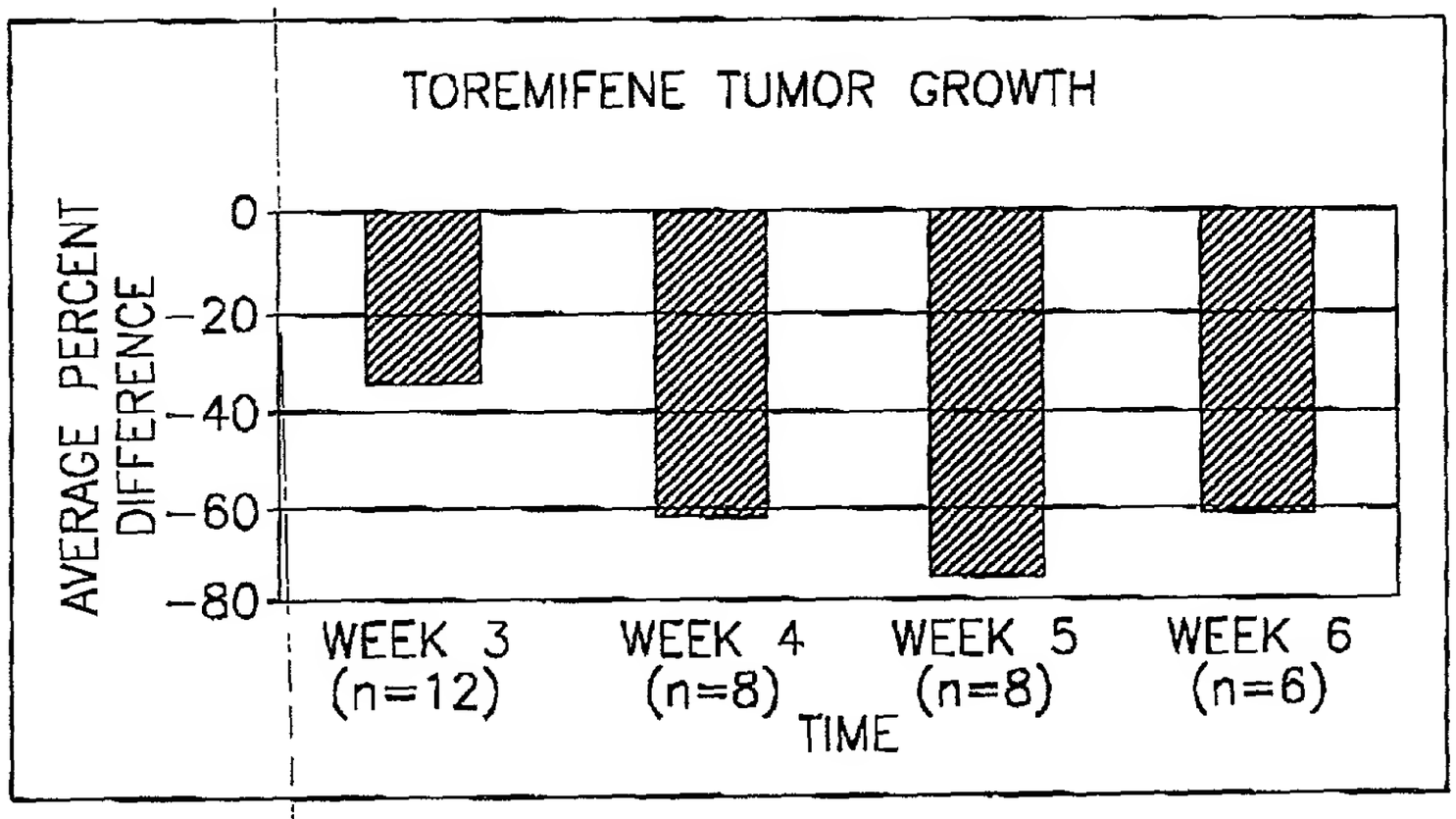


FIG.5



TIME AFTER TREATMENT	% CHANGE RELATIVE TO DAY 0
WEEK 3 (n=11)	9.44
WEEK 4 (n=8)	115.27
WEEK 5 (n=8)	271.71
WEEK 6 (n=8)	600.88



TIME AFTER TREATMENT	% CHANGE RELATIVE TO DAY 0
WEEK 3 (n=12)	-34.58
WEEK 4 (n=8)	-61.01
WEEK 5 (n=8)	-74.51
WEEK 6 (n=6)	-61.72

FIG.6

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below under my name.

I believe that I am the original, first and sole inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD FOR CHEMOPREVENTION OF PROSTATE CANCER

the Specification of which

☒ is attached hereto
☐ was filed on
as United States Application Number or PCT International Application No.
and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified Specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any provisional application filed in the United States in accordance with 35 U.S.C. §1.119(e), or any application for patent that has been converted to a Provisional Application within one (1) year of its filing date, or any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FILED APPLICATION(S)

APPLICATION NUMBER	COUNTRY	(DAY/MONTH/YEAR FILED)	PRIORITY CLAIMED

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in any prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a), which occurred

between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION NO.	FILING DATE (DAY/MONTH/YEAR)	STATUS - PATENTED, PENDING, ABANDONED
09/436,208	08-Nov-99	Pending
09/531,472	20-Mar-00	Pending

I hereby appoint as my attorney(s) and agent(s) Heidi M. Brun (Agent, Registration No. 35,104), or Daniel J. Swirsky (Agent, Registration No. 45, 148) or Mark S. Cohen (Attorney, Registration No. 42, 425) or Rochel L. Abboudi (Agent, Registration No. 44,490) or Suzanne Erez (Agent, Registration No. 46,688) said attorney(s) and agent(s) with full power of substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Direct all telephone calls to (703) 486-0600 and all facsimiles at (703) 486-0800.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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SIGNATURE OF INVENTOR _____

DATE _____

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FULL POST OFFICE ADDRESS: **same**

SIGNATURE OF INVENTOR _____

DATE _____